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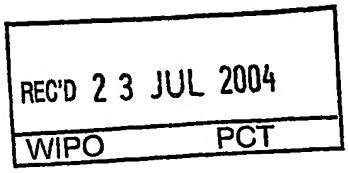

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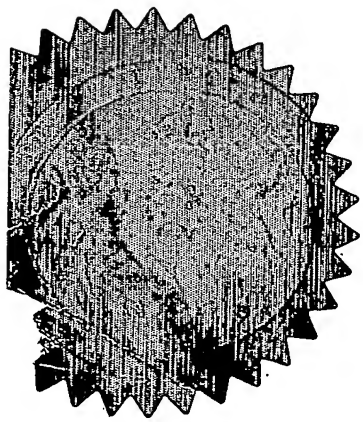

Signed Andrea Avery

Dated 6 April 2004

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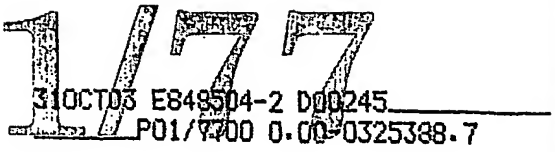

Request for grant of a patent

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1.	Your reference	**4-33445P1**
2.	Patent application number *(The Patent Office will fill in this part)*	0325388.7 — 30 OCT 2003
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	**NOVARTIS AG** **LICHTSTRASSE 35** **4056 BASEL** **SWITZERLAND** 712548700 5.
	Patent ADP number *(if you know it)*	
	If the applicant is a corporate body, give the country/state of its incorporation	**SWITZERLAND**
4.	Title of invention	**Organic Compounds**
5.	Name of your agent *(If you have one)*	**Bernard A. Marsh**
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	**Novartis Pharmaceuticals UK Limited** **Patents and Trademarks** **Wimblehurst Road** **Horsham, West Sussex** **RH12 5AB**
	Patents ADP number *(if you know it)*	**07181522002** ✓

6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country	Priority application number *(if you know it)*	Date of filing (day/month/year)

7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application	Date of filing (day/month/year)

8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.* *(see note (d))*	**Yes**

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Continuation sheets of this form	
Description	17
Claim(s)	1
Abstract	
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10. If you are also filing any of the following, state how many against each item.

Priority documents	
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Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application

Signature Date

Bernard A. Marsh 30th October 2003

12. Name and daytime telephone number of person to contact in the United Kingdom

Mr. Trevor Drew 01403 323069

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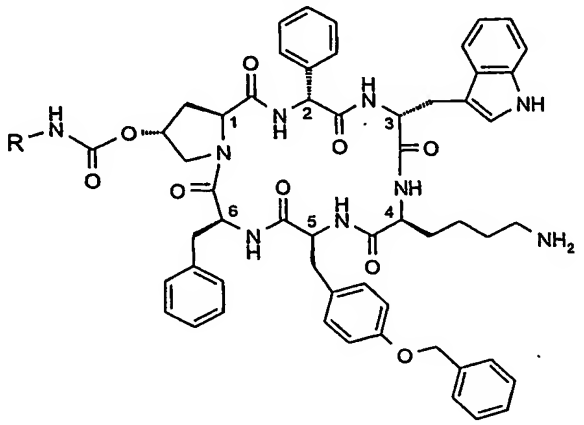
Organic Compounds

The present invention relates to somatostatin peptidomimetics, a process for their production and pharmaceutical preparations containing them.

More particularly the present invention provides the compound of formula I



wherein R is NR_1R_2-C_{2-6}alkylene or guanidine-C_{2-6}alkylene, and
each of $R_1$and R_2 independently is H or C_{1-4}alkyl,
in free form, in salt form or complex form, or in protected form.

Preferably R is NR_1R_2-C_{2-6}alkylene. A preferred compound of formula I is the compound wherein R is 2-amino-ethyl, also called cyclo[{4-(NH_2-C_2H_4-NH-CO-O-)Pro}-DPhg-DTrp-Lys-Tyr(4-Bzl)-Phe], and referred herein to as Compound A,
in free form, in salt or complex form or in protected form. Phg means -HN-CH(C_6H_5)-CO- and Bzl means benzyl.

A compound of formula I, e.g. Compound A, in protected form corresponds to above molecule wherein at least one of the amino groups is protected and which by deprotection leads to a compound of formula I, preferably physiologically removable. Suitable amino protecting groups are e.g. as disclosed in "Protective Groups in Organic Synthesis", T. W. Greene, J. Wiley & Sons NY (1981), 219-287, the contents of which being incorporated herein by reference. Example of such an amino protecting group is acetyl.

When a compound of formula I, e.g. Compound A, exists in complex form, it may conveniently be a compound of formula I bearing a chelating group on the side chain amino

group of Pro and complexed with a detectable or radiotherapeutic element. Compound A bearing a chelating group is referred to hereinto as conjugated Compound A.

Examples of chelating groups include e.g. those derived from poly-aminopolycarboxylic acids or anhydrides, e.g. those derived from non cyclic ligands e.g. diethylene triamine pentaacetic acid (DTPA), ethylene glycol-0,0'- bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), N,N'-bis(hydroxybenzyl)ethylenediamine-N,N'-diacetic acid (HBED) and triethylenetetramine hexaacetic acid (TTHA), those derived from substituted DTPA, e.g. p-isothiocyanato-benzyl-DTPA, those derived from macrocyclic ligands, e.g. 1,4,7,10-tetra-azacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA), 1,4,8,11-tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid (TETA), or 1,4,7,10-tetraazacyclotridecane-N,N',N'',N'''-tetra- acetic acid (TITRA).

The chelating group may be attached either directly or through a spacer to the side chain amino group of Pro. Suitable spacers include those known in the art, e.g. as disclosed in GB-A-2,225,579, for example the divalent residue of an amino-carboxylic acid, for example β-Ala or a divalent residue derived from 6-amino-caproic acid.

Preferred chelating groups are those derived from DTPA, DOTA or TETA. Chelating groups derived from DTPA or DOTA are most preferred.

By detectable element is meant any element, preferably a metal ion which exhibits a property detectable in vivo diagnostic techniques, e.g. a metal ion which emits a detectable radiation or a metal ion which is capable of influencing NMR relaxation properties. By radiotherapeutic element is meant any element which emits a radiation having a beneficial effect on the conditions to be treated.

Suitable elements include for example heavy elements or rare earth ions, e.g. as used in CAT scanning (Computer axial tomography), paramagnetic ions, e.g. Gd^{3+}, Fe^{3+}, Mn^{2+} and Cr^{2+}, fluorescent metal ions, e.g. Eu^{3+}, and radionuclides, e.g. a radiolanthanide, particularly a γ-emitting radionuclide, β-emitting radionuclide, α-emitting radionuclide, Auger-e^--emitting radionuclide or a positron-emitting radionuclide e.g. ^{68}Ga, ^{18}F or ^{86}Y.

Suitable γ-emitting radionuclides include those which are useful in diagnostic techniques. The γ-emitting radionuclides advantageously have a half-life of from 1 hour to 40 days, preferably from 5 hours to 4 days, more preferably from 12 hours to 3 days. Examples are radioisotopes from Gallium, Indium, Technetium, Ytterbium, Rhenium, Terbium, Lutetium, Thallium and Samarium e.g. ^{67}Ga, ^{111}In, ^{99m}Tc, ^{161}Tb, ^{169}Yb, ^{186}Re or ^{177}Lu.

Suitable β-emitting radionuclides include those which are useful in radiotherapeutic applications, for example ^{90}Y, ^{67}Cu, ^{186}Re, ^{188}Re, ^{169}Er, ^{121}Sn, ^{127}Te, ^{177}Lu, ^{143}Pr, ^{198}Au, ^{109}Pd, ^{165}Dy, ^{142}Pr or ^{153}Sm.

Suitable α-emitting radionuclides are those which are used in therapeutic treatments, e.g. ^{211}At, ^{212}Bi or ^{201}Tl.

Compounds of formula I, e.g. Compound A, may exist e.g. in free or salt form. Salts include acid addition salts with e.g. inorganic acids, polymeric acids or organic acids, for example with hydrochloric acid, acetic acid, lactic acid, aspartic acid, benzoic acid, succinic acid or pamoic acid. Acid addition salts may exist as mono- or divalent salts, e.g. depending whether 1 or 2 acid equivalents are added to the Compound A in free base form. Preferred salts are the lactate, aspartate, benzoate, succinate and pamoate including mono- and di-salts, more preferably the aspartate di-salt and the pamoate monosalt.

The conjugated compounds of formula I, e.g. conjugated Compound A, may additionally exist in salt forms obtainable with the carboxylic acid groups when present in the chelating group, e.g. alkali metal salts such as sodium or potassium, or substituted or unsubstituted ammonium salts.

The present invention also includes a process for the production of a compound of formula I, e.g. Compound A. It may be produced in analogy to known methods, for example:

a) cyclising a linear peptide in protected, polymer-bound or unprotected form in such a way that a compound of formula I, e.g. Compound A, is obtained and then optionally removing the protecting group(s),

b) to produce a conjugated compound of formula I, e.g. conjugated Compound A, linking together a chelating group and the compound of formula I, e.g. Compound A, in protected or unprotected form and then optionally removing the protecting group,

and recovering the compound of formula I, e.g. Compound A, or a conjugated compound of formula I, e.g. conjugated Compound, A thus obtained, in free form, in salt form or optionally complexed with a detectable or radiotherapeutic element.

It is generally not critical which amino acid is selected to be at the C-terminal position to start the peptide chain since the linear peptide will be cyclized, provided only that the sequence of amino acids in the linear peptide corresponds to that in the desired compound of formula I, e.g. Compound A. However there may be other factors which may prefer one starting amino acid over another. When a compound of formula I, e.g. Compound A, is prepared by solid phase synthesis, the first amino-acid is preferably attached to the resin, e.g. a commercially available polystyrene-based resin, through a suitable linker, e.g. a linker which is cleavable under mild conditions to keep the side chain protection intact, e.g. SASRIN or an optionally substituted trityl based linker, for example 4-(hydroxy-diphenyl-methyl)-benzoic acid wherein one the phenyl groups may optionally be substituted e.g. by Cl. The building up of the desired peptide chain may be effected in conventionnal manner, e.g. using amino-acid units wherein the terminal amino group is Fmoc-protected, the side chain amino groups where present being protected with a different amino protecting group, e.g. Boc or CBO. Preferably the linear peptide is cyclized in such a way to produce a bond between Tyr(4-Bzl)-OH and Phe, e.g.

Phe-{4-(NHR$_1$-C$_2$H$_4$-NH-CO-O-)Pro}-DPhg-DTrp(R$_2$)-Lys(ε-NHR$_3$)-Tyr(4-Bzl)-OH or a functional derivative thereof, wherein each of R$_1$, R$_2$ and R$_3$ is an amino protecting group. The cyclisation step a) may conveniently be performed according to known method, e.g. via an azide, an active ester, a mixed anhydride or a carbodiimide. Thereafter the protecting groups are removed, e.g. by cleavage e.g. with trifluoroacetic or by hydrogenation.

The cyclisation of the peptide may also be performed directly on the solid support, the first amino acid being in a Nα- and C-terminal protected form and attached through a side chain, e.g. ε-amino function of Lys or by backbone anchoring. The linear sequence is then synthesized following standard solid phase synthesis (SPPS) procedures. After cleavage of the C-terminal protection the peptide is cyclized e.g. as described above. Thereafter the cyclic peptide is cleaved from the resin and deprotected.

If desired, the lateral chain present on Pro may be introduced on the amino acid prior to or after the peptide cyclisation step a). Thus; Pro as a starting amino-acid or a starting linear or

cyclic peptide wherein in each case Pro is ring-substituted by OH, may be converted to provide a compound of formula I, e.g. Compound A, or the desired Pro unit or the corresponding linear peptide, respectively, wherein Pro is substituted by NHR_1-C_2H_4-NH-CO-O-.

The complexation of a conjugated compound of formula I, e.g. conjugated Compound A, may be performed by reacting the conjugated compound of formula I, e.g. the conjugated Compound A, with a corresponding detectable or radiotherapeutic element yielding compound, e.g. a metal salt, preferably a water-soluble salt. The reaction may be carried out by analogy with known methods, e.g. as disclosed in Perrin, Organic Ligand, Chemical Data Series 22. NY Pergamon Press (1982); in Krejcarit and Tucker, Biophys. Biochem. Res. Com. 77: 581 (1977) and in Wagner and Welch, J. Nucl. Med. 20: 428 (1979).

The following examples are illustrative of the invention. All temperatures are in °C.

Abbreviations:

AcOH	= acetic acid
Boc	= tert.-butoxy-carbonyl
Bzl	= benzyl
CBO	= carbobenzoxy
DIPCI	= N,N'-diisopropylcarbodiimide
DIPEA	= diisopropylethylamine
DMF	= dimethylformamide
DPPA	= diphenylphosphorylazide
Fmoc	= fluorenylmethoxycarbonyl
HOBT	= 1-hydroxybenzotriazole
Osu	= N-hydroxysuccinimide
TFA	= trifluoroacetic acid
THF	= tetrahydrofuran

Example 1: Cyclo[{4-(NH_2-C_2H_4-NH-CO-O-)Pro}-DPhg-DTrp-Lys-Tyr(4-Bzl)-Phe]

a) Synthesis of Fmoc-Pro(4-OCO-NH-CH_2-CH_2-NH-Boc)-OH

L-hydroxyproline methylester hydrochloride is reacted with Fmoc-OSu in aqueous 1.0 N sodium carbonate/THF at room temperature. After completion of the reaction, Fmoc-Pro(4-OH)-OMe is isolated by precipitation. Fmoc-Pro(4-OH)-OMe is then added dropwise into a solution of trisphosgene (0.6 eq.) in THF to give a chlorocarbonate intermediate. After 1h dimethylaminopyridine (1.0 eq.) and N-Boc-diaminoethane (6.0 eq.) are added and the reaction is stirred at room temperature. After completion of the reaction, the solvent is removed *in vacuo* and the resulting Fmoc-Pro(4-OCO-NH-CH_2-CH_2-NH-Boc)-OMe is extracted from a two phase system of ethyl acetate/0.1 M HCl to give crude product (MH^+ = 554) which is purified by crystallization from ethyl acetate. The methyl ester is then cleaved to the free acid by treatment with 1N NaOH in dioxane/water and the product Fmoc-Pro(4-OCO-NH-CH_2-CH_2-NH-Boc)-OH is purified on silica gel, $[(M+Na)]^+$ = 562).

b) H-Phe-Pro(4-OCO-NH-CH_2-CH_2-NH-Boc)-DPhg-DTrp(Boc)-Lys(Boc)-Tyr(Bzl)-OH
Commercially available Fmoc-Tyr(Bzl)-O-CH_2-Ph(3-OCH_3)-O-CH_2-Polystyrene resin (SASRIN-resin, 2.4 mM) is used as starting material and carried through a standard protocol consisting of repetitive cycles of Nα-deprotection (Piperidine/DMF, 2:8), repeated washings with DMF and coupling (DIPCI: 4.8 mM/HOBT: 6mM, DMF). The following amino acid-derivatives are sequentially coupled: Fmoc-Lys(Boc)-OH, Fmoc-DTrp(Boc)-OH, Fmoc-DPhg-OH, Fmoc-Pro(4-OCO-NH-CH_2-CH_2-NH-Boc)-OH, Fmoc-Phe-OH. Couplings (2 eq. amino acids) are continued or repeated until completion, i.e. until complete disappearance of residual amino groups which is monitored by a negative 'Kaiser' Ninhydrin test. Before cleavage of the completely assembled protected linear peptide from its resin support the Nα-Fmoc protection from the last residue is removed.

c) H-Phe-Pro(4-OCO-NH-CH_2-CH_2-NH-Boc)-DPhg-DTrp(Boc)-Lys(Boc)-Tyr(Bzl)-OH
After washings with CH_2Cl_2, the peptide-resin is transferred into a column or a stirred suction filter and the peptide fragment is cleaved and eluted with a short treatment with 2% TFA in CH_2Cl_2 within 1 h. The eluate is immediately neutralized with a saturated $NaHCO_3$ solution. The organic solution is separated and evaporated and the side chain protected precursor (MH^+ = 1366) is cyclized without further purification.

d) cyclo[-Pro(4-OCO-NH-CH_2-CH_2-NH_2)-DPhg-DTrp-Lys-Tyr(Bzl)-Phe-], trifluoroacetate
The above linear fragment is dissolved in DMF (4 mM), cooled to minus 5°C and treated with 2 eq. DIPEA then 1.5 eq. of DPPA and stirred until completion (ca. 20h) at 0-4°C. The

solvent was almost completely removed in vacuo; the concentrate is diluted with ethyl acetate, washed with $NaHCO_3$, water, dried and evaporated in vacuo.

For deprotection the residue is dissolved at 0°C in TFA/H_2O 95:5 (ca. 50 mM) and stirred in the cold for 30 min. The product is then precipitated with ether containing ca. 10 eq. HCl, filtered, washed with ether and dried. In order to completely decompose remaining Indole-N carbaminic acid the product is dissolved in 5% AcOH and lyophilized after 15 h at ca. 5°C.. Preparative RP-HPLC is carried out on a C-18 10 µm STAGROMA column (5-25 cm) using a gradient of 0.5% TFA to 0.5% TFA in 70% acetonitrile. Fractions containing the pure title compound are combined, diluted with water and lyophilized. The lyophilisate is dissolved in water followed by precipitation with 10% Na_2CO_3 in water. The solid free base is filtered of, washed with water and dried in vacuum at room temperature. The resulting white powder is directly used for the different salts.

Example 2: Cyclo[{4-(NH_2-C_2H_4-NH-CO-O-)Pro}-DPhg-DTrp-Lys-Tyr(4-Bzl)-Phe] in salt form

a. Acetate

Conversion to the acetate salt form is carried out using an ion-exchange resin (e.g. AG 3-X4). MS (ESI): m/z 524.5 $[M+2H]^{2+}$

$[\alpha]_D^{20}$ = -41.6°; c=0.56; AcOH 95%; T = 20C; 589.3 nm

b. Aspartate

Conversion to the mono- or di-aspartate is obtained by reacting 1 equivalent of the compound of Example 1 with 1 or 2 equivalent of aspartic acid in a mixture of acetonitrile/water 1:3. The resulting mixture is frozen and lyophilized.

The di-aspartate may also be obtained by dissolving the compound of Example 1 in water/acetonitrile 4:1, filtering, loading on a an ion-exchange resin, e.g. BioRad AG4X4 column, and eluting with water/acetonitrile 4:1. The eluate is concentrated, frozen and lyophilized.

c. Benzoate

Conversion to the benzoate may be obtained by dissolving the compound of Example 1 with 2 equivalents of benzoic acid in a mixture of acetonitrile/water 1:2. The resulting mixture is frozen and lyophilized.

d. Pamoate

1 equivalent of the compound of Example 1 is dissolved together with 1 equivalent of embonic acid in a mixture of acetonitrile/THF/water 2:2:1. The resulting mixture is frozen and lyophilized.

Example 3: Cyclo[{4-(DOTA-NH-C_2H_4-NH-CO-O-)Pro}-DPhg-DTrp-Lys-Tyr(4-Bzl)-Phe

a) cyclo[-Pro(4-OCO-NH-CH_2-CH_2-NH_2)-DPhg-DTrp-Lys(Cbo)-Tyr(Bzl)-Phe-], trifluoroacetate

The compound is synthesised in the same way like cyclo[-Pro(4-OCO-NH-CH_2-CH_2-NH_2)-DPhg-DTrp-Lys(Cbo)-Tyr(Bzl)-Phe-], trifluoroacetate by using Fmoc-Lys(Cbo)-OH instead of Fmoc-Lys(Boc)-OH.

b) 400 mg commercially available DOTA x $2H_2O$ (SYMAFEX – France) is dissolved in 20 ml water. After addition of 20 ml DMF, 170 mg cyclo[-Pro(4-OCO-NH-CH_2-CH_2-NH_2)-DPhg-DTrp-Lys(CBO)-Tyr(Bzl)-Phe-], together with 190 mg DCCI and 60 mg N-hydroxysuccinimide are added. The resulting suspension is kept at room temperature for 72 hours. After filtration, the solvent is removed under reduced pressure and the remaining crude is purified on silica gel (DCM/MeOH/$HOAc_{50\%}$ 8/2/0.25 --> 7/3/1 as mobile phase).

c) For deprotection the above DOTA – conjugate is treated with 5 ml trifluoroacetic acid/thioanisole (9/1) for two hours at room temperature. After that the solution is poured into a mixture of 100 ml diethylether + 5 ml 3N HCl/diethylether and the resulting precipitate ias isolated by filtration. Purification is performed on silica gel using DCM/MeOH/$HOAc_{50\%}$ 7/4/2 --> 7/5/4 as mobile phase. Analytically pure endproduct is obtained after a desalting step using a 0.1% TFA to 0.1% TFA in 90 % CH_3CN gradient on a RP_{18}-HPLC column (Spherisorb 250 x 4.6 mm). MH^+: 1434.7

Compounds of formula I, e.g. Compound A, in free form or in the form of pharmaceutically acceptable salts and complexes exhibits valuable pharmacological properties as indicated in <u>in vitro</u> and <u>in vivo</u> tests and is therefore indicated for therapy.

More particularly, compounds of formula I, e.g. Compound A, exhibit an interesting binding profile for human somatostatin receptors (hsst). 5 somatostatin receptor subtypes, sst1, sst2, sst3, sst4 and sst5 have been cloned and characterized. hsst1, hsst2 and hsst3 and their sequences have been disclosed by Y. Yamada et al. in Proc. Nat. Acad. Sci., 89, 251-255 (1992). hsst4 and its sequence have been disclosed by L. Rohrer et al. in Proc. Acad. Sci., 90, 4196-4200 (1993). hsst5 and its sequence have been described by R. Panetta et al. in Mol. Pharmacol. 45, 417-427, 1993.

The binding assays may be carried out as disclosed hereunder using membranes from cell lines expressing selectively and stably hsst1, hsst2, hsst3, hsst4 or hsst5, e.g. CHO or COS cells.

Membranes are prepared according to known methods, e.g. as disclosed by C. Bruns et al. in Biochem. J., 1990, 65, page 39-44. Membranes prepared from hsst selective cell lines, e.g. CHO or COS cells stably expressing hsst1 or hsst2 or hsst3 or hsst4 or hsst5 are incubated in triplicate in a total volume of 300 µl at 22°C for 30 min with increasing concentrations of [^{125}I-Tyr^{11}]-SRIF-14 in 10 mmol/l Hepes buffer (pH 7.6) containing 0.5% BSA. The incubation is terminated by rapid filtration and the filters are counted in a counter. Specific binding is total binding minus non-specific binding in the presence of 1 µmol/l somatostatin-14. The experiments are carried out in triplicate. The affinity constant (K_D) and number of binding sites are calculated using appropriate statistics and graphical programs.

Compounds of formula I, e.g. Compound A, have no significant binding affinity in the above binding assays towards hsst1, hsst2 and hsst4, a low binding affinity towards hsst3 and a good binding affinity towards hsst5 expressed as an IC_{50} value in the nMolar range (IC_{50} = concentration for half-maximal inhibition in a competition binding assay using [^{125}I-Tyr^{11}]-SRIF-14 as specific radioligand).

IC_{50}

	hsst1	hsst2	hsst3	hsst4	hsst5
Compound A	>1000	>1000	22 nM	840 nM	0.53nM

Compounds of formula I, e.g. Compound A, show GH-release inhibiting activity as indicated by the inhibition of GH release in vitro from cultured pituitary cells. For example, anterior pituitary glands from adult male rats are cut into small pieces and dispersed using 0.1 % trypsin in 20 mM HEPES buffer. The dispersed cells are cultured for four days in MEM (Gibco) supplemented with 5 % fetal calf serum, 5% horse serum, 1 mM $NaHCO_3$, 2.5 nM dexamethasone, 2.5 mg/ml insulin and 20 U/ml Pen/Strep. On the day of the experiment the attached cells are washed two times with Krebs-Ringer medium buffered with 20 mM HEPES and supplemented with 5 mM glucose and 0.2 % BSA. Subsequently the cells are incubated for three hours with Compound A in the presence of $3x10^{-10}$ M growth hormone releasing factor. The amount of growth hormone released into the medium is measured by RIA.

Compounds of formula I, e.g. Compound A, inhibit the release of growth hormone (GH) in rats. Compound A is administered s.c. to anaesthetized rats. Blood is collected after decapitation 1 h after administration of the compound. The duration of action is estimated on the basis of the inhibition of basal GH secretion 6 h after drug treatment. Hormone levels are measured by RIA 1h and 6h after treatment. The ID_{50}-value for the inhibition of the hormone secretion is determined graphically (log-probit) for each experiment and the resulting values are averaged logarithmically. In this in vivo model Compound A inhibits growth hormone release.

Compounds of formula I, e.g. Compound A, are also useful in the treatment of tumors which are hsst3 and/or hsst5 receptor positive, as indicated in proliferation tests with various cancer cell lines bearing hsst3 and/or hsst5.

Compounds of formula I, e.g. Compound A, are accordingly useful for the prevention or treatment of disorders with an aetiology comprising or associated with the presence or activation of hsst3 and/or hsst5, e.g. disorders or diseases associated with excess GH-secretion e.g. in the treatment of acromegaly or for the treatment of malignant cell proliferative diseases, e.g. cancer tumors bearing hsst3 and/or hsst5, e.g. as disclosed hereunder for the complexed conjugated Compound A.

For all the above indications the required dosage will of course vary depending upon, for example, the host, the mode of administration and the severity of the condition to be

treated. In general, however, satisfactory results are obtained by administration in the order of from 1 µg to 0.7 mg/kg/day of compound of formula I, e.g. Compound A. An indicated daily dosage for patients is in the range from about 2 µg to about 50 mg, preferably about 0.01 to about 40 mg, e.g. about 0.01 to about 3 mg s.c. of the compound conveniently administered in divided doses up to 3 times a day in unit dosage form containing for example from about 0.5 µg to about 25 mg, e.g. from about 2 µg to 20 mg, for example from 2 µg to 1.5 mg of compound of formula I, e.g. Compound A.

The compounds of formula I, e.g. Compound A may be administered in free form or in pharmaceutically acceptable salt form or complexes. Such salts and complexes may be prepared in conventional manner and exhibit the same order of activity as the free compound. The present invention also provides a pharmaceutical composition comprising a compound of formula I, e.g. Compound A, in free base form or in pharmaceutically acceptable salt form or complex form, together with one or more pharmaceutically acceptable diluent or carrier. Such compositions may be formulated in conventional manner. The compounds of formula I, e.g. Compound A may also be administered in sustained release form, e.g. in the form of implants, microcapsules, microspheres or nanospheres comprising e.g. a biodegradable polymer or copolymer, in the form of a liposomal formulation, or in the form of an autogel, e.g. a solid or semi-solid composition capable of forming a gel after interaction with patient's body fluids.

The compounds of formula I, e.g. Compound A, or a pharmaceutically acceptable salt or complex thereof may be administered by any conventional route, for example parenterally e.g. in form of injectable solutions or suspensions (including e.g. the sustained release form as indicated above), orally using a conventional absorption enhancer, in a nasal or a suppository form or topically, e.g. in the form of an ophthalmic liquid, gel, oinment or suspension preparation, e.g a liposomal, microsphere or nanosphere formulation, e.g. for instillation or subconjunctival or intra- or peri-ocular injections.

In accordance with the foregoing the present invention further provides:

1. a compound of formula I, e.g. Compound A, or a pharmaceutically acceptable salt or complex thereof for use as a pharmaceutical;

2. A method of preventing or treating diseases or disorders as herein indicated in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula I, e.g. Compound A, or a pharmaceutically acceptable salt or complex thereof; or

3. a compound of formula I, e.g. Compound A, or a pharmaceutically acceptable salt or complex thereof for use in the preparation of a pharmaceutical composition for use in any method as defined under 2. above.

A conjugated compound of formula I, e.g. Compound A, or a pharmaceutically acceptable salt thereof is useful either as an imaging agent, e.g. visualisation of hsst3 and/or hsst5 receptor positive tissues and cells e.g. hsst3 and/or hsst5 receptor positive tumors and metastases, inflammatory or autoimmune disorders exhibiting somatostatin receptors, tuberculosis or organ rejection after transplantation, when complexed with a detectable element, e.g. a γ- or positron-emitting nuclide, a fluorescent metal ion or a paramagnetic ion, e.g. ^{111}In, ^{161}Tb, ^{177}Lu, ^{86}Y, ^{68}Ga Eu^{3+}, Gd^{3+}, Fe^{3+}, Mn^{2+} or Cr^{2+}, or as a radiopharmaceutical for the treatment in vivo of hsst3 and/or hsst5 receptor positive tumors and metastases, rheumatoid arthritis and severe inflammation conditions when complexed with an α- or β-emitting nuclide or a nuclide with Auger-e^--cascades, e.g. ^{90}Y, ^{161}Tb, ^{177}Lu, ^{211}At, ^{213}Bi or ^{201}Tl, as indicated by standard tests.

In particular, it is observed that the conjugated Compound A binds to somatostatin receptors with pKi values of from about 8 to 10. Compound of Example 3 complexed with e.g. ^{111}In, ^{88}Y, ^{90}Y or ^{177}Lu binds in the nM range to the respective sst sub-types in accordance with the binding profile of Compound A.

The affinity of a conjugated compound of formula I, e.g conjugated Compound A, and its complexes for hsst3 and/or hsst5 receptors can also be shown by in vivo testing, according to standard test methods, e.g. as disclosed in GB-A-2,225,579. For example the compound of Example 3 complexed with e.g. ^{111}In, ^{88}Y, ^{90}Y or ^{177}Lu, gives a

significant tumor accumulation 4 hours after injection into mice or rats bearing an exocrine pancreatic tumor expressing hsst5 receptors.

After administration of a conjugated compound of formula I, e.g. conjugated Compound A, in complexed form, e.g. radiolabelled with ^{111}In, ^{177}Lu, ^{86}Y or ^{161}Tb, at a dosage of from 1 to 5 µg/kg labelled with 0.1 to 5 mCi radionuclide, preferably 0.1 to 2 mCi, the tumor site becomes detectable.

The conjugated compound of formula I, e.g. conjugated Compound A, when radiolabelled with an α- or β-emitting radionuclide or a nuclide with Auger-e⁻-cascades exhibits an antiproliferative and/or cytotoxic effect on tumor cells bearing hsst3 and/or hsst5 receptors, e.g. as indicated in nude mice tests.

Nude mice are inoculated with hsst5 bearing tumor cells. When tumors have reached a volume of 1 to 2 cm^3 animals are randomized into control and treatment groups. A conjugated compound of formula I, e.g. conjugated Compound A in complexed form is administered by i.p. or i.v. injections. Doses up to 40mCi/kg are given per mouse. The size of the tumors is determined with a caliper as disclosed above. For statistical calculations Student's t-test is applied. In this test, transient tumor shrinkage is observed after one week and tumor growth is delayed for two weeks upon a single application of the compound of Example 3 complexed with ^{90}Y or ^{177}Lu. In contrast the control groups showed continuous tumor growth with a volume doubling time of about seven days.

Accordingly, in a series of specific or alternative embodiments, the present invention also provides:

4. Use of a conjugated compound of formula I, e.g. conjugated Compound A, complexed with a detectable element for in vivo detection of hsst3 and/or hsst5 positive cells and tissues, e.g. hsst3 or hsst5 positive tumors and metastasis, in a subject and recording the localisation of the receptors targeted by said complex;

5. A method for in vivo detection of hsst3 and/or hsst5 positive tissues and cells, e.g. hsst3 or hsst5 positive tumors and metastasis, in a subject comprising administering to said subject a conjugated compound of formula I, e.g. conjugated

Compound A, complexed with a detectable element, or a pharmaceutically acceptable salt form, and recording the localization of the receptors targeted by said complex.

The conjugated compound of formula I, e.g. conjugated Compound A, in complexed form for use as an imaging agent may be administered e.g. intravenously, e.g. in the form of injectable solutions or suspensions, preferably in the form of a single injection. The radiolabelling may preferably be performed shortly before administration to a subject.

In animals an indicated dosage range may be from 0.01 to 1 μg/kg of a conjugated compound of formula I, e.g. conjugated Compound A, complexed with 0.02 to 0.5 mCi γ-emitting radionuclide. In larger mammals, for example humans, an indicated dosage range may be from 1 to 100 $\mu g/m^2$ conjugated Compound A complexed e.g. with 1 to 100 mCi/m^2 detectable element, e.g. ^{111}In, ^{86}Y or ^{177}Lu.

6. Use of a conjugated compound of formula I, e.g. conjugated Compound A, complexed with an α- or β-emitting nuclide or a nuclide with Auger-e^--cascades, for in vivo treatment of hsst3 and/or hsst5 positive tumors and metastases.

7. A method for in vivo treatment of hsst3 and/or hsst5 positive tumors and metastases, e.g. for treating invasiveness of such tumors or symptoms associated with such tumor growth, in a subject in need of such treatment which comprises administering to said subject a therapeutically effective amount of a conjugated compound of formula I, e.g. conjugated Compound A, complexed with an α- or β-emitting nuclide or a nuclide with Auger-e^-cascades.

8. Use of a conjugated compound of formula I, e.g. conjugated Compound A, or a pharmaceutically acceptable salt thereof in the manufacture of an imaging agent or a radiopharmaceutical composition.

Dosages employed in practising the radiotherapeutic use of the present invention will of course vary depending e.g. on the particular condition to be treated, for example the known radiotoxicity to normal organs expressing hsst5, the volume of the tumor and the

therapy desired. In general, the dose is calculated on the basis of pharmacokinetik and radioactivity distribution data obtained in to healthy organs and based on the observed target uptake. A β-emitting complex of a conjugated compound of formula I, e.g. conjugated Compound A, may be administered repeatedly e.g. over a period of 1 to 3 months.

In animals an indicated dosage range may be from 20 to 100 μg/kg conjugated compound of formula I, e.g. conjugated Compound A, complexed with 15 to 70 mCi of an α- or β-emitting nuclide or a nuclide with Auger-e^-cascades, e.g. ^{90}Y, ^{177}Lu or ^{161}Tb. In larger mammals, for example humans, an indicated dosage range may be from 1 to 100 $\mu g/m^2$ conjugated compound of formula I, e.g. conjugated Compound A, complexed e.g. with 1 to 100 mCi/m^2 of an α- or β-emitting nuclide or a nuclide with Auger-e^--cascades, e.g. ^{90}Y, ^{177}Lu or ^{161}Tb.

A conjugated compound of formula I, e.g. conjugated Compound A, in complexed form for use as a radiotherapeutic agent may be administered by any conventional route, e.g. intravenously, e.g. in the form of injectable solutions. It may also be administered advantageously by infusion, e.g. an infusion over 15 to 60 min. Depending on the site of the tumor, it may be administered as close as possible to the tumor site, e.g. by means of a catheter. The present invention also provides a pharmaceutical composition comprising a conjugated compound of formula I, e.g. conjugated Compound A, in free base form or in pharmaceutically acceptable salt form or complexed with a detectable or radiotherapeutic agent, together with one or more pharmaceutically acceptable diluent or carrier.

A compound of formula I or a conjugated compound of formula I, e.g. Compound A or the conjugated Compound A, in complexed form may be suitable for imaging or treating hsst3 and/or hsst5 expressing or accumulating such as pituitary tumors, e.g. adenomas or prolactinomas, gastro-enteropancreatic tumors, carcinoids, central nervous system, breast, prostatic (including advanced hormone-refractory prostate cancer), ovarian or colonic tumours, small cell lung cancer, malignant bowel obstruction, paragangliomas, kidney cancer, skin cancer, neuroblastomas, pheochromocytomas, medullary thyroid carcinomas, myelomas, lymphomas, Hodgkins and non-Hodgkins lymphomas, bone

tumours and metastases thereof, as well as autoimmune or inflammatory disorders, e.g. rheumatoid arthritis, Graves disease or other inflammatory eye diseases.

According to a further aspect of the invention, there is provided a pharmaceutical composition comprising a conjugated compound of formula I, e.g. conjugated Compound A, or a complex thereof together with one or more pharmaceutically acceptable carriers or diluents therefor. Such compositions may be manufactured in conventional manner and may be presented, e.g. for imaging, in form of a kit comprising two separate dosages, one being the radionuclide and the other the conjugated compound of formula I, e.g. conjugated Compound A, with instructions for mixing them. For radiotherapy, the conjugated compound of formula I, e.g conjugated Compound A, in complexed form may preferably be in the form of a hot liquid formulation.

A compound of formula I optionally conjugated, e.g. Compound A or a conjugated Compound A, in complexed form may be administered as the sole active ingredient or in conjuction with, e.g. as an adjuvant to, other drugs. For example, a compound of formula I, e.g. Compound A, may be used in combination with an immunosuppressive agent, e.g. a calcineurin inhibitor, e.g. cyclosporin A, Isa Tx247 or FK 506; a mTOR inhibitor, e.g. rapamycin, CCI779, ABT578 or 40-O-(2-hydroxyethyl)-rapamycin; an ascomycin having immunosuppressive properties, e.g. ABT-281, ASM981, etc.; corticosteroids; cyclophosphamide; azathioprene; methotrexate; leflunomide; mizoribine; mycophenolic acid or a salt thereof, e.g. Myfortic^{R}; mycophenolate mofetil; 15-deoxyspergualine or an immunosuppressive homologue, analogue or derivative thereof; an accelerating lymphocyte homing agent, e.g. FTY720; immunosuppressive monoclonal antibodies, e.g., monoclonal antibodies to leukocyte receptors; e.g., MHC, CD2, CD3, CD4, CD7, CD8, CD25, CD28, CD40, CD45, CD58, CD80, CD86 or to their ligands; other immunomodulatory compounds, e.g. a recombinant binding molecule having at least a portion of the extracellular domain of CTLA4 or a mutant thereof, e.g. an at least extracellular portion of CTLA4 or a mutant thereof joined to a non-CTLA4 protein sequence, e.g. CTLA4Ig (for ex. designated ATCC 68629) or a mutant thereof, e.g. LEA29Y; adhesion molecule inhibitors, e.g. LFA-1 antagonists, ICAM-1 or -3 antagonists, VCAM-4 antagonists or VLA-4 antagonists. A compound of formula I, e.g. Compound A may also be used in combination with an anti-inflammatory agent, a GH secretagogue receptor modulating agent, e.g. ghrelin or hexarelin, a GH receptor antagonist, e.g. pegvisomant,

A compound of formula I optionally conjugated, e.g. Compound A or a conjugated Compound A, in complexed form may also be used in combination with an antiproliferative agent, e.g a chemotherapeutic drug, e.g. paclitaxel, gemcitabine, cisplatinum, doxorubicin, 5-fluorouracil or taxol, a hormonal agent or antagonist, e.g. an anti-androgen or mitoxantrone (especially in the case of prostate cancer), or an antiestrogen, like letrozole (especially in the case of breast cancer), an antimetabolite, a plant alkaloid, a biological response modifier, preferably a lymphokine or interferons, an inhibitor of protein tyrosine kinase and/or serine/threonine kinase, or an agent with other or unknown mechanism of action, e.g.any epothilone or epothilone derivative, or a mTOR inhibitor, e.g. as indicated above..

Where a compound of formula I optionally conjugated, e.g. Compound A or a conjugated Compound A, in complexed form is administered in conjunction with another drug, dosages of the co-administered drug will of course vary depending on the type of co-drug employed, on the specific drug employed, on the condition to be treated, and so forth. The terms "co-administration" or "combined administration" or the like as utilized herein are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

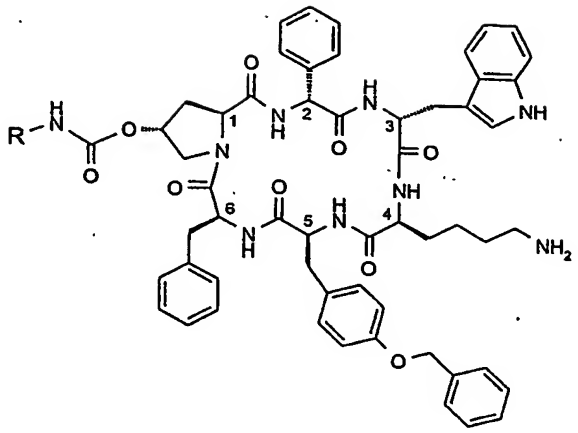
In accordance with the foregoing the present invention provides in a yet further aspect:

9. A pharmaceutical combination comprising a) a first agent which is a compound of formula I optionally conjugated, e.g. Compound A or a conjugated Compound A, in complexed form and b) a co-agent, e.g. as defined above.

10. A method as defined above comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective amount of a compound of formula I optionally conjugated, e.g. Compound A or a conjugated Compound A in complexed form, and a second drug substance, said second drug substance being, e.g. as indicated above.

CLAIMS

1. A compound of formula I



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wherein R is NR_1R_2-C_{2-6}alkylene or guanidine-C_{2-6}alkylene, and
each of $R_1$and R_2 independently is H or C_{1-4}alkyl,
in free form, in salt form or complex form, or in protected form, e.g. cyclo[{4-(NH_2-C_2H_4-NH-CO-O-)Pro}-DPhg-DTrp-Lys-Tyr(4-Bzl)-Phe],
in free form, in salt or complex form or in protected form,
a process for its preparation, its use as a pharmaceutical, method of treatment using such a compound or a pharmaceutically acceptable salt or complex thereof and pharmaceutical compositions comprising such a compound or a pharmaceutically acceptable salt or complex thereof, substantially as hereinbefore defined and/or described.